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論文題目：Molecular characterization of cellulosic polysaccharides by size-exclusion chromatography equipped with a multi-angle light scattering detector (SEC-MALS)
（SEC-MALS 法によるセルロース系多糖の分子特性解析）

In this study, the SEC-MALS (size-exclusion chromatography equipped with a multi-angle light scattering detector) method was applied to various cellulosic polysaccharides, and their molecular properties, i.e. molecular mass (M), molecular mass distribution, molecular conformation and chain stiffness (or the Kuhn segment length: l_K) were evaluated (chapter 2 and 3).

Along this procedure, some kinds of intermolecular structures (e.g. aggregates, insoluble residues and branching/cross-linking structures) were found for some cases, then their causes, origins, growth/dissolution conditions and other properties were studied (chapter 4, 5 and 6).

In chapter 2, the lithium chloride/1,3-dimethyl-2-imidazolidinone (LiCl/DMI) solvent system was adopted as a mobile phase of SEC analysis of cellulose, and validity of the method was examined using MALS and ^{13}C-NMR analysis. The results indicate that ① 8% LiCl/DMI is a true solvent for cellulose; ② no aggregates exist in the LiCl/DMI solutions of the cellulose samples used; ③ cellulose molecules dissolving in 1% LiCl/DMI are separated orderly depending on their M or radii of gyration (R_g) by the SEC system; ④ cellulose molecules once dissolved in LiCl/DMI keep their M and molecularly-dispersed state for a long time (6 months or longer).

In chapter 3, the SEC-MALS-QELS (quasi-elastic light scattering) method was applied to cellulose and cellulose tricarbanilate (CTC) samples using LiCl/amide solvents and THF as the mobile phases. CTC was used as a reference because its

solution behavior and molecular properties had been thoroughly characterized. Molecular conformations of cellulose and CTC in the solvents were then compared and discussed on the basis of the relationships between R_g, the hydrodynamic radii (R_h; obtained by QELS) and degrees of polymerization (DP) or the contour lengths (L). The Benoit-Doty theory for wormlike polymer chains was applied to the R_g vs. L data, and the theoretical curves with the l_K of around 18 nm were found to fit the data of both cellulose and CTC molecules in the solvents. This indicates that cellulose and CTC molecules have conformations essentially identical to each other, regardless of the kinds of substituents, solvents or dissolution mechanisms, and thus the dominant factor determining the molecular conformations of cellulosic polysaccharides in their dilute solutions is the characteristics of the main chains consisting of β-1,4-glucoside linkages.

In chapter 4, the SEC-MALS method was applied to chitosan samples having different average M and degree of N-acetylation (F_A) values. The influence of these parameters on dispersion states of chitosan molecules in aqueous media was preliminarily examined by conformation analysis. It was shown that even a slight amount of residual N-acetyl groups (as low as 2% F_A) caused the presence of aggregates, which gave critical effects on the data for the conformation analysis. Such aggregates could not be removed by common techniques such as preparative centrifugation (7740G, 10 min), microfiltration (0.1 μm) or lowering ionic strength of the solution. On the other hand, completely deacetylated samples had no such structures, and gave consistent data regardless of their M values. Therefore, complete deacetylation is highly recommended in advance of the conformation analysis of chitosan, although some depolymerization is unavoidable during the repeated deacetylation treatments.

In chapter 5, various cellulose and wood pulp samples including softwood kraft pulp (SKP) were dissolved in 8% LiCl/DMI and 8% LiCl/N,N-dimethylacetamide (LiCl/DMAc), and the obtained solutions were subjected to SEC-MALS. Although SKP was not completely soluble in 8% LiCl/DMAc, 8% LiCl/DMI gave a clear solution of SKP without centrifugation. Moreover, conformation analysis of them revealed that some compact structures like branches or cross-linkages other than molecularly-dispersed cellulose were present in the high M fraction of SKP dissolved in 1% LiCl/DMI. Since no such structure was found in the soluble fraction of SKP in 8% LiCl/DMAc, the insoluble fraction of SKP in 8% LiCl/DMAc exactly contributes to the branching and cross-linking structures in LiCl/DMI. Bleaching or removal of residual lignin did not affect these structures, while they were lost by acidic treatments or mannanase treatments. Thus, the key factor of the structure was found to be glucomannan in softwood.

In chapter 6, various wood pulp samples including SKP and softwood holocellulose were dissolved in 8% LiCl/DMI, and the obtained solutions were analyzed by means of a size exclusion chromatograph attached with photodiode array and multi-angle laser light scattering detectors (SEC-PDA-MALS) using 1% LiCl/DMI as a mobile phase to collect information concerning DP, DP distributions, distributions of residual lignin and their UV-VIS absorption patterns. Changes in DP of SKP during the conventional bleaching sequence were evaluated as well as those of the residual lignin present in there. About half of the residual lignin in softwood unbleached and bleached kraft pulps was present in the high-DP polysaccharide fractions, which

occupied approximately 90% of the pulps. Some characteristic differences in the UV-VIS absorption pattern were observed between kraft pulps bleached at oxygen and chlorine stages. *DP*, *DP* distribution of polysaccharides and distribution of residual lignin were clearly different between softwood unbleached kraft pulp, softwood unbleached sulfite pulp, softwood holocellulose and hardwood unbleached kraft pulp. The UV-VIS absorption patterns due to the residual lignin were also different between these pulp and holocellulose samples.